

What is Claimed:

1. A modified follicle stimulating hormone (FSH), containing an amino acid sequence which differs from the wild-type FSH, said modified FSH comprising a modified  $\alpha$ -subunit, wherein the potency of said modified FSH is increased by at least about a ten fold as compared to wild type FSH.
2. The modified FSH of claim 1, wherein the potency of said modified FSH is increased by at least about 30 fold as compared to wild type FSH.
3. The modified FSH of claim 1, wherein the potency of said modified glycoprotein hormone is increased by at least about 50 fold as compared to wild type FSH.
4. The modified FSH of claim 1, wherein said modified FSH is selected from the group consisting of human, bovine, equine, porcine, ovine, murine, primate, fish, etc.
5. The modified FSH of claim 4, wherein said modified FSH is human.
6. The modified FSH of claim 5, wherein said modified  $\alpha$ -subunit comprises at least two basic amino acids at positions corresponding to positions 13, 14, 16, 17, 20, 21, 22, 66, 68, 73, 74 and 81 of SEQ ID No. 1.
7. The modified FSH of claim 6, wherein said basic amino acids of the  $\alpha$ -subunit are at positions 14 and 66.
8. The modified FSH of claim 7, wherein said basic amino acids are E14R and N66R.
9. The modified FSH of claim 6, wherein said basic amino acids of the  $\alpha$ -subunit are at positions 14 and 73.
10. The modified FSH of claim 9, wherein said basic amino acids are E14R and G73R.
11. The modified FSH of claim 6, wherein said basic amino acids of the  $\alpha$ -subunit are at positions 16 and 20.
12. The modified FSH of claim 11, wherein said basic amino acids are P16R and Q20R.

13. The modified FSH of claim 6, wherein said basic amino acids of the  $\alpha$ -subunit are at positions 20 and 21.
14. The modified FSH of claim 13, wherein said basic amino acids are Q20R and P21R.
15. The modified FSH of claim 6, said  $\alpha$ -subunit further comprising a third basic amino acid at a position selected from the group consisting of positions 13, 14, 16, 17, 20, 21, 22, 66, 68, 73, 74 and 81.
16. The modified FSH of claim 15, wherein said basic amino acids of the  $\alpha$ -subunit are at positions 16, 20 and 21.
17. The modified FSH of claim 16, wherein said basic amino acids are P16R, Q20R and P21R.
18. The modified FSH of claim 15, wherein said basic amino acids of the  $\alpha$ -subunit are at positions 14, 20 and 73.
19. The modified FSH of claim 18, wherein said basic amino acids are E14R, Q20R and G73R.
20. The modified FSH of claim 15, wherein said basic amino acids of the  $\alpha$ -subunit are at positions 66, 73 and 81.
21. The modified FSH of claim 20, wherein said basic amino acids are N66K, G73K and A81K.
22. The modified FSH of claim 15, wherein said basic amino acids of the  $\alpha$ -subunit are at positions 14, 66 and 73.
23. The modified FSH of claim 22, wherein said basic amino acids are E14R, N66R and G73R.
24. The modified FSH of claim 15, wherein said basic amino acids of the  $\alpha$ -subunit are at positions 14, 21 and 73.
25. The modified FSH of claim 24, wherein said basic amino acids are E14R, P21R and G73R.

26. The modified FSH of claim 15, said  $\alpha$ -subunit further comprising a fourth basic amino acid at a position selected from the group consisting of positions 13, 14, 16, 17, 20, 21, 22, 66, 68, 73, 74 and 81.
27. The modified FSH of claim 26, wherein said basic amino acids of the  $\alpha$ -subunit are at positions 13, 14, 16 and 20.
28. The modified FSH of claim 27, wherein said basic amino acids are Q13R, E14R, P16R and Q20R.
29. The modified FSH of claim 28, wherein said basic amino acids are Q13K, E14K, P16K and Q20K.
30. The modified FSH of claim 26, said  $\alpha$ -subunit further comprising a fifth basic amino acid at a position selected from the group consisting of positions 13, 14, 16, 17, 20, 21, 22, 66, 68, 73, 74 and 81.
31. The modified FSH of claim 30, wherein said basic amino acids of the  $\alpha$ -subunit are at positions 14, 20, 21, 66 and 73.
32. The modified FSH of claim 31, wherein said basic amino acids are E14R, Q20R, P21R, N66R and G73R.
33. The modified FSH of claim 30, wherein said basic amino acids of the  $\alpha$ -subunit are at positions 14, 16, 20, 66 and 73.
34. The modified FSH of claim 33, wherein said basic amino acids are E14R, P16R, Q20R, N66R and G73R.
35. The modified FSH of claim 30, said  $\alpha$ -subunit further comprising a sixth basic amino acid at a position selected from the group consisting of positions 13, 14, 16, 17, 20, 21, 22, 66, 68, 73, 74 and 81.
36. The modified FSH of claim 35, wherein said basic amino acids of the  $\alpha$ -subunit are at positions 13, 14, 16, 20, 66 and 73.

37. The modified FSH of claim 36, wherein said basic amino acids are Q13K, E14K, P16K, Q20K, N66K and G73K.
38. The modified FSH of claim 35, wherein said basic amino acids of the  $\alpha$ -subunit are at positions 14, 16, 20, 21, 66 and 73.
39. The modified FSH of claim 38, wherein said basic amino acids are E14R, P16R, Q20R, P21R, N66R and G73R.
40. The modified FSH of claim 6, wherein said basic amino acids are selected from the group consisting of lysine and arginine.
41. The modified FSH of claim 1, further comprising a modified  $\beta$ -subunit.
42. The modified FSH of claim 41, wherein said modified  $\beta$ -subunit comprises at least one basic amino acid at a position corresponding to positions 2, 4, 14, 63, 64, 67 and 69 of SEQ ID No. 2.
43. The modified FSH of claim 42, wherein said basic amino acid is E4R.
44. A nucleic acid encoding the modified FSH  $\alpha$ -subunit of claim 1.
45. A vector comprising the nucleic acid of claim 44, wherein the vector is suitable for expressing the nucleic acid.
46. A host cell comprising the vector of claim 45, wherein the host cell is suitable for expressing the nucleic acid.
47. The modified FSH of claim 6, wherein said modified FSH has less than five amino acid substitutions in said  $\alpha$ -subunit in positions other than positions 13, 14, 16, 17, 20, 21, 22, 66, 68, 73, 74 and 81.
48. The modified FSH of claim 6, wherein said modified FSH has less than four amino acid substitutions in said  $\alpha$ -subunit in positions other than positions 13, 14, 16, 17, 20, 21, 22, 66, 68, 73, 74 and 81.

49. The modified FSH of claim 6, wherein said modified FSH has less than three amino acid substitutions in said  $\alpha$ -subunit in positions other than positions 13, 14, 16, 17, 20, 21, 22, 66, 68, 73, 74 and 81.
50. The modified FSH of claim 6, wherein said modified FSH has less than two amino acid substitutions in said  $\alpha$ -subunit in positions other than positions 13, 14, 16, 17, 20, 21, 22, 66, 68, 73, 74 and 81.
51. The modified FSH of claim 6, wherein said modified FSH has complete amino acid sequence identity with the corresponding wild-type FSH in said  $\alpha$ -subunit in positions other than positions 13, 14, 16, 17, 20, 21, 22, 66, 68, 73, 74 and 81.
52. The modified FSH of claim 1, wherein the plasma half-life is increased as compared to wild type FSH.
53. The modified FSH of claim 52, wherein said modified FSH further comprises at least one sequence with a potential glycosylation site selected from the group consisting of a sequence comprising a N-glycosylation site and a sequence comprising an O-glycosylation site.
54. The modified FSH of claim 53, wherein said at least one sequence with a potential glycosylation recognition site is an N-terminal extension on said  $\alpha$  chain.
55. The modified FSH of claim 54, wherein said N-terminal extension is selected from the group consisting of ANITV (SEQ ID No. 3) and ANITVNITV (SEQ ID No. 4).
56. The modified FSH of claim 53, wherein said at least one sequence with a potential glycosylation recognition site is a substitution in said  $\beta$  chain.
57. The modified FSH of claim 56, wherein said substitution is selected from the group consisting of Y58N and V78N.
58. The modified FSH of claim 52, wherein said modified FSH is pegylated.
59. The modified FSH of claim 52, wherein said modified FSH is altered to increase the number of negatively charged residues within the molecule to increase plasma half-life.

60. The method of claim 59, wherein said negatively charged residues are selected from the group consisting of glutamate and aspartate.
61. The modified FSH of claim 60, wherein said alteration is selected from the group consisting of alpha subunit substitutions A85E and A85D.
62. The modified FSH of claim 59, wherein said alteration is an insertion of an amino acid sequence containing one or more negatively charged residues into said modified FSH.
63. The modified FSH of claim 62, wherein said insertion is selected from the group consisting of GEFT (SEQ ID No. 5) and GEFTT (SEQ ID No. 6).
64. The modified FSH of claim 63, wherein said insertion is in the alpha subunit.
65. The modified FSH of claim 64, wherein said insertion is accompanied by a deletion of one or more amino acids.
66. The modified FSH of claim 64, wherein said insertion is selected from the group consisting of APD-GEFT-VQDC (SEQ ID No. 7) and APD-GEFTT-QDC (SEQ ID No. 8).
67. A method of assisting reproduction in a subject comprising administering an assisting amount of the modified FSH of claim 1.
68. A method of diagnosing and/or treating a condition associated with a glycoprotein hormone activity in a patient comprising administering an effective amount of the modified FSH of claim 1 to the patient.
69. The method of claim 68, wherein the condition is ovulatory dysfunction.
70. The method of claim 68, wherein the condition is a luteal phase defect.
71. The method of claim 68, wherein the condition is unexplained infertility.
72. The method of claim 68, wherein the condition is male factor infertility.
73. The method of claim 68, wherein the condition is time-limited conception.
74. The method of claim 68, wherein the patient demonstrates low FSH receptor expression in growing follicles.

75. The method of claim 68, wherein the patient demonstrates low FSH receptor sensitivity.
76. The method of claim 68, wherein the patient demonstrates FSH receptor binding deficiency.
77. The method of claim 68, wherein said patient demonstrates FSH receptor coupling deficiency.
78. The method of claim 68, where said patient demonstrates male pattern baldness.
79. The method of claim 68, wherein said patient demonstrates deficient levels of testosterone production.
80. The method of claim 68, wherein said patient demonstrates pituitary failure or injury.
81. The method of claim 68, wherein the condition is ovarian carcinoma.
82. The method of claim 68, wherein the condition is testicular carcinoma.
83. A method of reducing hyperstimulation syndrome in a patient undergoing assisted reproduction therapy, comprising
- (a) administering an assisting amount of a first modified FSH according to claim 1 wherein the plasma half-life of said first modified FSH is increased as compared to wild type FSH, and
  - (b) subsequently administering an assisting amount of a second modified FSH according to claim 1 wherein the plasma half-life of said second modified FSH is decreased as compared to said first modified FSH, wherein ovarian hyperstimulation is reduced as compared to when the same patient is administered only said first modified FSH during assisted reproduction therapy.
84. A method of improving the quality of oocytes in an animal comprising:
- administering an effective amount of a superactive follicle stimulating hormone to said animal,

wherein said superactive follicle stimulating hormone contains an  $\alpha$ -subunit with a basic amino acid at one or more positions selected from the group consisting of positions 13, 14, 16, and 20.

85. A method of claim 84, wherein said improvement in the quality of oocytes is characterized by an improvement in fertilization rate of oocytes in the animal as compared to a like animal receiving the same amount of recombinant wild type FSH.

86. A method of claim 85, wherein the rate of fertilized oocytes increases at least about 10 % as a result of administration of said superactive follicle stimulating hormone at the maximally effective dose for oocyte number.

87. A method of claim 84, wherein said improvement in the quality of oocytes is characterized by an improvement in blastocyst formation rate per fertilized oocyte in the animal compared to a like animal receiving the same amount of recombinant wild type FSH.

88. A method of claim 87, wherein rate of blastocyst formation increases at least about 10 % as a result of administration of said superactive follicle stimulating hormone at the maximally effective dose for oocyte number.

89. A method of claim 84, wherein said improvement in the quality of oocytes is characterized by an improvement in total number of embryos per fertilized oocyte of the animal compared to a like animal receiving the same amount of recombinant wild type FSH.

90. A method of claim 89, wherein the total number of embryos per fertilized oocyte increases at least about 10 % as a result of administration of said superactive follicle stimulating hormone at the maximally effective dose for oocyte number.

91. A method of claim 84, wherein the basic amino acid of the  $\alpha$ -subunit is an arginine, a lysine, a histidine, or a modification thereof.

92. A method of claim 84, wherein the basic amino acid of the  $\alpha$ -subunit is positively charged at a neutral pH.

93. A method of claim 84, wherein the superactive FSH contains an arginine at positions 13, 14, 16, and 20.



94. A method of claim 84, wherein the superactive follicle stimulating hormone contains a lysine at positions 13, 14, 16, and 20.
95. A method of claim 84, wherein the alpha-subunit contains a modification to prolong half-life.
96. A method of claim 95, wherein the modification to prolong half-life is an ANITV extension.
97. A method of claim 84, wherein the superactive follicle stimulating hormone is a human superactive follicle stimulating hormone.
98. A method of claim 84, wherein the animal is selected from the group consisting of human, mouse, rat, primate, rabbit, pig, cow, horse, sheep, and dog.
99. A method of claim 84, wherein the superactive follicle stimulating hormone is administered by injection or ingestion.
100. A method of claim 84, further comprising administering hCG.
101. A method of claim 84, wherein the superactive follicle stimulating hormone contains a wildtype alpha-subunit.
102. A method of claim 84, wherein the superactive follicle stimulating hormone contains an alpha-subunit with a basic amino acid at position 4.
103. A method of claim 102, wherein the basic amino acid is an arginine, a lysine, a histidine, or a modification thereof.
104. A method of inducing superovulation in an animal comprising:
- administering an effective amount of superactive follicle stimulating hormone to said animal,
- wherein said superactive follicle stimulating hormone contains an alpha-subunit with a basic amino acid at one or more positions selected from the group consisting of positions 13, 14, 16, and 20.

105. A method of claim 104, wherein superovulation is characterized by an increase in oocyte number as compared to a like animal receiving the same amount of recombinant wild type FSH.
106. A method of claim 105, wherein the average oocyte number increases at least about 10 % as a result of administration of said superactive follicle stimulating hormone at the maximally effective dose for oocyte number.
107. A method of claim 104, wherein the basic amino acid of the  $\alpha$ -subunit is an arginine, a lysine, a histidine, or a modification thereof.
108. A method of claim 104, wherein the basic amino acid of the  $\alpha$ -subunit is positively charged at a neutral pH.
109. A method of claim 104, wherein the superactive FSH contains an arginine at positions 13, 14, 16, and 20.
110. A method of claim 104, wherein the superactive follicle stimulating hormone contains a lysine at positions 13, 14, 16, and 20.
111. A method of claim 104, wherein the alpha-subunit contains a modification to prolong half-life.
112. A method of claim 111, wherein the modification to prolong half-life is an ANITV (SEQ ID No. 3) extension.
113. A method of claim 104, wherein the superactive follicle stimulating hormone is a human superactive follicle stimulating hormone.
114. A method of claim 104, wherein the animal is selected from a group consisting of a human, mouse, rat, primate, rabbit, pig, cow, horse, sheep, and dog.
115. A method of claim 104, wherein the superactive follicle stimulating hormone is administered by injection or ingestion.
116. A method of claim 104, further comprising administering hCG.

117. A method of claim 104, wherein the superactive follicle stimulating hormone contains a wildtype alpha-subunit.
118. A method of claim 104, wherein the superactive follicle stimulating hormone contains an alpha-subunit with a basic amino acid at position 4.
119. A method of claim 118, wherein the basic amino acid is an arginine, a lysine, a histidine, or a modification thereof.
120. A method of enhancing *in vitro* fertilization comprising:
- administering an effective amount of superactive follicle stimulating hormone to said animal,
- wherein said superactive follicle stimulating hormone contains an alpha-subunit with a basic amino acid at one or more positions selected from the group consisting of positions 13, 14, 16, and 20.
121. A method of claim 120, wherein the basic amino acid of the alpha-subunit is an arginine, a lysine, a histidine, or a modification thereof.
122. A method of claim 120, wherein the basic amino acid of the alpha-subunit is positively charged at a neutral pH.
123. A method of claim 120, wherein the superactive FSH contains an arginine at positions 13, 14, 16, and 20.
124. A method of claim 120, wherein the superactive follicle stimulating hormone contains a lysine at positions 13, 14, 16, and 20.
125. A method of claim 120, wherein the alpha-subunit contains a modification to prolong half-life.
126. A method of claim 125, wherein the modification to prolong half-life is an ANITV (SEQ ID No. 3) extension.
127. A method of claim 120, wherein the superactive follicle stimulating hormone is a human superactive follicle stimulating hormone.

128. A method of claim 120, wherein the animal is selected from a group consisting of a human, mouse, rat, primate, rabbit, pig, cow, horse, sheep, and dog.
129. A method of claim 120, wherein the superactive follicle stimulating hormone is administered by injection or ingestion.
130. A method of claim 120, further comprising administering hCG.
131. A method of claim 120, wherein the superactive follicle stimulating hormone contains a wildtype alpha-subunit.
132. A method of claim 120, wherein the superactive follicle stimulating hormone contains a alpha-subunit with a basic amino acid at position 4.
133. A method of claim 132, wherein the basic amino acid is an arginine, a lysine, a histidine, or a modification thereof.
134. The modified FSH of claim 1, wherein the plasma half-life is decreased as compared to wild type FSH.
135. The modified FSH of claim 1, wherein there is a decrease in absorption compared to wild type FSH.
136. The modified FSH of claim 1, wherein there is an increase in absorption compared to wild type FSH.
137. The modified FSH of claim 1, wherein there is an increase in binding affinity to a FSH receptor compared to wild type FSH.